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Risk Assessment

Application of the European Non-native Species in Aquaculture Risk Analysis Scheme to evaluate *Micropterus nigricans* in China

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Abstract

Largemouth bass *Micropterus nigricans* has caused a wide range of invasive impacts and is listed among the top 100 of the World's Worst Invasive Alien Species by IUCN. Yet it has been widely introduced around the world due to sport fishing needs and its importance for aquaculture. It also has been promoted most vigorously in the past five years in China, where the production of *M. nigricans* exceeded 90% of the total production of perches in 2019. In this study, the invasion risk of *M. nigricans* was assessed using AS-ISK andfound to he high in the middle and lower reaches of the Changjiang River. The European Non-native Species in Aquaculture Risk Analysis Scheme (ENSARS) was therefore used to evaluate the full invasive risk of *M. nigricans* in the same area. The results suggest that *M. nigricans* carries an overall medium risk of invasion in China, with every module suggesting a medium risk. Confidence ranks for the overall scores fell into the range of 2.0–2.5, which suggests a high confidence level. This study is meant to be a trial for further use of the ENSARS scheme in China, especially for potential non-native aquacultural species.

Key words: aquacultural species, risk assessment, freshwater fish, AS-ISK, ENSARS

Introduction

Aquaculture is currently recognized as an important sector of the food industry globally (Madsen and Stauffer 2024). Global aquaculture production totaled 122.6 million tonnes in 2020 and retained its growth rate under the spread of the COVID-19 pandemic (FAO 2022). At the same time, the development of finfish farming has become the most important driver for the introduction of non-native species worldwide (Casal 2006). The aquaculture of non-native species poses persistent risks of biological invasions, especially in countries and regions with large production volumes but weak security controls (Kang et al. 2022).

China's aquaculture industry has experienced a rapid development in recent years, consistently contributing over 60% to the world's total output. In 2021, China's aquacultural production reached 53.94 million tonnes, accounting for 80.6% of the nation's total aquatic products (FAO 2022). In the same year, the total production of freshwater fishes was 26.4 million tonnes, representing 83% of the total freshwater aquaculture production (China Fishery Statistical Yearbook 2022). China, being a major aquacultural nation, has a high proportion of the cultivation of non-native species worldwide, reaching up to 25% (Lin et al. 2015).

In China, black basses (*Micropterus* spp.) account for the second-largest distinctive freshwater fishes after tilapias (*Orechromis* spp. and *Tilapia* spp.) (China Fishery Statistical Yearbook 2022; Gu et al. 2022). In 2019, the production of all finfish in the country reached 477,808 tonnes, and surged to 702,000 tonnes in 2021. The majority of this production comes from the aquaculture of non-native largemouth bass *Micropterus nigricans* (Lacepède, 1802) (China Fishery Statistical Yearbook 2022). Of note, the scientific name of the largemouth bass was recently changed from *Micropterus salmoides* to *Micropterus nigricans* (Kim et al. 2022). Black basses have gained popularity in aquaculture and among consumers in China due to their fast growth, strong resistance to disease and parasites, short breeding cycle, high yield, and tasty flesh (Bai and Li 2013). In recent years, they have become one of the most widely recommended non-native freshwater fish species for aquaculture in China (Chen et al. 2020). In the late 1970s, *M. nigricans*, after successful artificial breeding in 1983, was introduced for aquaculture to Guangdong Province and, subsequently, to Jiangsu, Shandong and Zhejiang Provinces, Shanghai, and other regions of China. From 2016 to 2018, Zhejiang Province included *M. nigricans* as one of the most recommended aquacultural species. The breeding area and operational scale of *M. nigricans* has continuously increased, making it one of the most successful non-native species in Chinese aquaculture (Hussein et al. 2020; Gui et al. 2018).

Since the breakthrough in artificial breeding technology for *M. nigricans* in 2017, the aquaculture of this species has been further promoted nationwide. Currently, *M. nigricans* has been discovered in various natural water bodies in southern China, such as the Beijiang River in the Pearl River Basin (Gu et al. 2012), the Beipan River in Guizhou Province (Chen et al. 2013), the Suijiang River and Baixi River in the lower reaches of the Jinshajiang River Basin at the border of Yunnan and Sichuan provinces (Gao et al. 2013), the Wujiang River as a tributary of the Changjiang River (Chen et al. 2013), and Dianchi Lake in Yunnan Province (Xiao et al. 2020). In some areas, *M. nigricans* has already established self-sustaining populations, and its discovery in the wild is most likely a result of sport fishing, charity release, or escapes during aquaculture and transportation processes (Chen et al. 2013; Xiao et al. 2020; Yu et al. 2024). However, no risk assessment was conducted in China prior to the aquaculture of this species.

Apart from China, *M. nigricans* has been also frequently introduced worldwide mostly as a game fish or for aquaculture (Pereira and Vitule 2019). This species is currently found in all six inhabited continents, although the natural range is from the St. Lawrence River to northern Mexico and the east coast of North America (Brown et al. 2009; Page and Burr 2011; Stauffer et al. 2016). Where *M. nigricans* has been introduced either for sport fishing or aquaculture, propagule pressure (i.e. the number of individuals introduced with a certain frequency) is usually high because of frequent release and escape into natural streams, where the species becomes an aggressive predator of native species (Minckley 1973; Britton and Gozlan 2013; Pereira and Vitule 2019). These factors may increase the species' probability of introduction, escape, establishment, and dispersal, leading to its successful invasion worldwide (Britton et al. 2010; Britton and Gozlan 2013; Kang et al. 2022).

Development, structure, and applications of the European Non-native Species in Aquaculture Risk Analysis Scheme

In this study, the European Non-native Species in Aquaculture Risk Analysis Scheme (ENSARS) was used to conduct a risk assessment of *M. nigricans*. With the implementation of the European Sustainable Aquaculture Development Strategy, the European Commission enacted Council Regulation (EC) No 708/2007 concerning the use of alien and locally absent species in aquaculture, aiming to promote the sustainability of this food sector. In response to this regulation, the ENSARS was developed to identify and assess the potential risks associated with the use of non-native species in aquaculture (Copp et al. 2009, 2016a). This scheme originated from the European and Mediterranean Plant Protection Organization Decision Support Scheme for Pest Risk Analysis (EPPO 2012). It provides guidance for evaluating the potential risks of introduction of a non-native species into a predefined risk assessment area, including the potential impacts on native species and the ecosystem, and serves as a basis for subsequent management and policy enactment.

The ENSARS consists of eight modules, with seven being the (core) assessment modules: *entry*, *pre-screening*, *pathway*, *facility*, *organism*, *infectious agent*, and *socio- economic* (Copp et al. 2016b). These modules are structured based on a common format consisting of a series of questions that the assessor(s) must answer. Each answer is associated with a confidence level. Due to the interconnected information among the modules, during the assessment process these can be used either individually or in combination (Copp et al. 2016a).

The application of the ENSARS as a risk assessment tool can be compared to that of the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016b) as a risk screening tool. The AS-ISK is applicable to all aquatic species (excluding parasites and pathogens) in any eco-region of the world. It has been applied in various countries and climate zones, including China, for screening a variety of aquatic organisms (Vilizzi et al. 2021). As per the ENSARS, its initial application involved a trial evaluation of the 24 non-native species listed in Annex IV of Council Regulation No 708/2007 (EU 2007). In that study, all 24 species were assessed under the Biological Risk Assessment Module, with comprehensive evaluations conducted for wels catfish *Silurus glanis* Linnaeus, 1758 and red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Copp et al. 2016b). The ENSARS has been utilized in Türkiye and Brazil to assess the potential risks associated with the aquaculture of non-native striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Oreochromis niloticus* (Linnaeus, 1758) (Tarkan et al. 2020; de Camargo et al. 2022, respectively). The toolkit has also been applied in China for the assessment of marble goby *Oxyeleotris marmorata* (Bleeker, 1852) (Wei et al. 2024).

Consistent with the efforts in non-native species management in China in recent years, the purpose of this study was to conduct a comprehensive risk assessment of *M. nigricans* in aquaculture using the ENSARS, with a suggestion of improvement of this scheme for future applications worldwide.

Methodology

Risk assessment area

The risk assessment area in this study is the middle and lower reaches of the Changjiang River (Yangtze River drainage). The main stem of the river is 1,893 km from Yichang to the river mouth and with an area of 800,000 km^2 spanning Hubei, Hunan, Anhui, Jiangxi, Zhejiang, and Jiangsu Provinces, and Shanghai (Figure 1). Annual average air temperature in the area is 14–18 °C, and the highest and lowest temperatures are recorded in July and January (27–28 °C and 0–5.5 °C, respectively). Annual precipitation is 800–1600 mm (Liu et al. 2017). Water temperatures in the area are predicted based on air temperature (Erickson and Stefan 1996; Nan et al. 2005). Accordingly, the highest and lowest water temperatures occur in July (27.7–28.6 °C) and January (3.5–8.4 °C) (Liu et al. 2017). By 2050, the temperature of the Changjiang River is predicted to increase by 2.2 °C due to the impact of greenhouse gases, and by 2100 by 4.5 °C due to the impact of sulfide aerosol (Xu et al. 2004). The average precipitation in the research area has no obvious predicted change (Xu et al. 2004).

Pre-screening with AS-ISK

Two experts (B. Kang and H. Wei) with knowledge of the fishes of the middle and lower Changjiang River conducted the assessment of the ENSARS *pre-screening* module (AS- ISK) for *M. nigricans*. The AS-ISK consists of 55 questions,

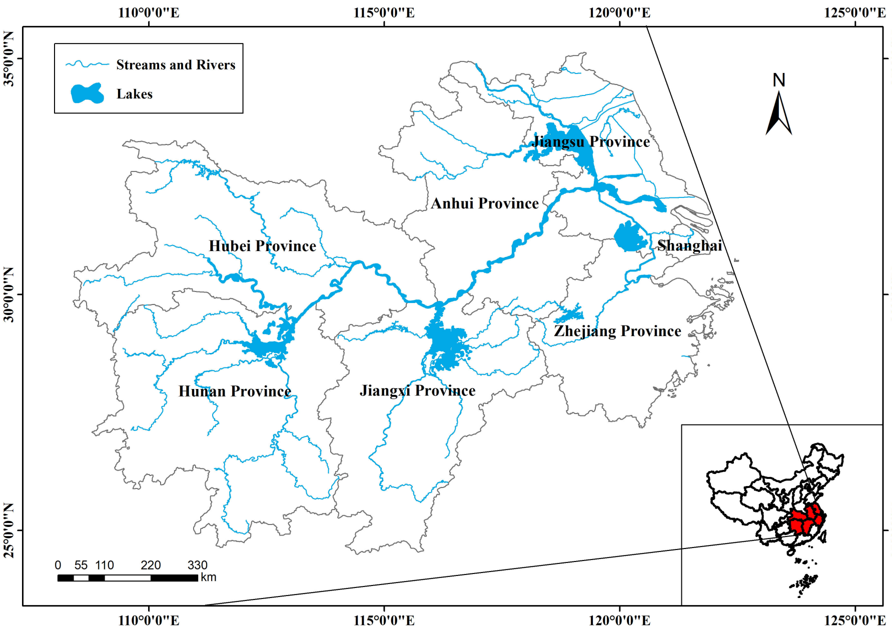

Figure 1. Middle and lower reaches of the Changjiang River of China. The main stein of the river is of 1,893 km from Yichang (Hubei Province) to the mouth spanning an area of 800,000 km^2 that encompasses Hubei, Hunan, Anhui, Jiangxi, Zhejiang and Jiangsu Provinces, and Shanghai.

with each assessor providing for each question a response, confidence level, and justification. Based on the assessor(s)' responses, each species receives a Basic Risk Assessment (BRA) score and a BRA + Climate Change Assessment (CCA) score (Vilizzi et al. 2021). The calibrated threshold of 26 was used in this study to distinguish among species carrying a "high" and "medium" risk of invasiveness for Shanghai in the lower Changjiang River (Yu et al. 2024). If the BRA or BRA+CCA scores of the screened species are ≧ threshold, then the species is ranked as high risk; if the scores are ≧ 1 and < threshold, then the species is ranked as medium risk; if the scores are < 1, then the species is ranked as low risk (Vilizzi et al. 2022).

Full assessment with ENSARS

Three experts (B. Kang, H. Wei and D. Xu) with knowledge also of the aquaculture of *M. nigricans* completed the five full assessment modules (i.e. *organism*, *infectious agent*, *facility*, *pathway*, and *socio-economic*). The assessors responded to each question with a score of 0–4, and the answer was supported by a justification (based on research publications with or without English titles and abstract, grey literature, i.e. survey reports, government documents, and news or expert opinion). The overall mean scores and mean scores for each category (low risk: 0–0.7; moderately low risk: 0.8–1.5; medium risk: 1.6–2.3; moderately high risk: 2.4–3.1; high risk: 3.2–4.0) along with the confidence levels (0 = low; 1 = moderate; 2 = high; 3 = very high) were obtained following Copp et al. (2016a).

Table 1. Pre-screening results for *Micropterus nigricans* in the middle and lower reaches of the Changjiang River using the Aquatic Species Invasiveness Screening Kit. The screening was done by two experts B. Kang and H. Wei. Risk outcomes are based on a threshold of 26 (after Yu et al. 2024) to distinguish between medium risk and high risk species.

	Mean scores
BRA	26.0
BRA Outcome	High
BRA+CCA	36.0
BRA+CCA Outcome	High
Score partition	
1. Domestication/Cultivation	3.0
2. Climate, distribution and introduction risk	2.0
3. Invasive elsewhere	6.0
B. Biology/Ecology	15.0
4. Undesirable (or persistence) traits	7.5
5. Resource exploitation	5.0
6. Reproduction	−1.0
7. Dispersal mechanisms	0.0
8. Tolerance attributes	3.5
C. Climate change	10.0
9. Climate change	10.0
Sectors affected Commercial	
Environmental	12.5
Species or population nuisance traits	17.5
Confidence BRA+CCA	
BRA	0.7
CCA	0.4

Results

The Results of the ENSARS *pre-screening* module based on the AS-ISK identified *M. nigricans* as posing a high risk of being invasive in the middle and lower reaches of the Changjiang River, with a mean BRA score of 26.0 (Table 1; Supplementary material Table S1). The higher mean BRA+CCA score of 36.0 suggested an increase in the invasion risk of *M. nigricans* based on climate change predictions. The high-risk outcome from the *pre-screening* module pointed to the need of a full, follow-up risk assessment, which was conducted with the ENSARS (Tables S2–S6).

The risk category of all five modules of the ENSARS ranked *M. nigricans* as a medium-risk species. The overall score of the *organism* module was the highest at 2.3. The top three highest scores were given to the *establishment* section of the *socio-economic* module, the *farming* section of the *pathway* module, and the *establishment* section of the *organism* module (Table 2). The top two lowest scores were given to the *introduction* sections of the *organism* module and the *infectious agent* module.

The confidence rank of the overall means in all modules was high (2.0–2.5). Compared to the sections in the other four assessment modules that received a high confidence rank (> 2.0), all sections in the *socio-economic* module received a lower confidence rank (≦ 2.0). All sections in the *organism*, *infectious agent*, *facility*, and *pathway* modules attracted a high confidence rank. The lowest confidence rank was attributed to the eradication costs section and to the impact at a wider local or national scale of the *socio-economic* module.

Table 2. European Non-native Species in Aquaculture Risk Analysis Scheme applied to *Micropterus nigricans* in the middle and lower reaches of the Changjiang River with risk category and overall mean scores and confidence levels (in parentheses) for the *organism, infectious agent, facility, pathway* and *socio-economic* modules. Score interval risk categories after Copp et al. (2016a); low risk: 0–0.7, moderately low risk: 0.8–1.5, medium risk: 1.6–2.3, moderately high risk: 2.4–3.1, high risk: 3.2–4.0. Mean confidence levels were derived from the following confidence ranks attributed to each assessment response: 0 = low, 1 = moderate, 2 = high and 3 = very high.

Module	Risk category	Overall	Introduction	Establishment	Dispersal	Impact
Pathway			Import	Farming	Destination use	
	Medium	2.0 (2.5)	1.6 (2.5)	2.5 (2.4)	1.9 (2.6)	
Facility			Release of target organism	Release of non- target organism		
	Medium	1.9 (2.4)	2.1 (2.4)	1.6 (2.4)		
Organism	Medium	2.3 (2.5)	1.4 (2.6)	2.3 (2.4)	2.4 (2.3)	1.7 (2.6)
Infectious agent	Medium	2.0 (2.3)	1.5 (2.7)	2.1 (2.3)	2.0 (2.1)	1.6 (2.2)
Socio-economic			Market impacts	Eradication costs	Impact at a wider local/national scale	
	Medium risk	1.8 (2.0)	1.6 (2.0)	2.7 (1.9)	1.6 (1.9)	

Discussion

In this study, the application of the ENSARS to assess the level of invasiveness of *M. nigricans* in the middle and lower reaches of the Changjiang River suggested a medium risk for all modules with a high confidence level. An exception were the establishment and dispersal sections of the *socio-economic* module, which had moderate confidence level. This may be attributable to the lack of references for the socio-economic questions for this species in China.

The *pathway* module assessed the potential risks of *M. nigricans* escaping through various pathways into the wild within the risk assessment area. This module consists of three parts dealing with the three different steps in the production chain: import, farming, and destination or use of the product. Because of the absence of intramuscular spines and a moderate market price, *M. nigricans*, like other centrarchids, has become popular for family meals and banquets. The development of pre-made dishes has also expanded the market for this fish. In 2017, breakthroughs in artificial compound feed technology for *M. nigricans* replaced the traditional method of feeding with fresh fish, leading to a quick increase in the number of fish farmers (AF 2022). Consequently, the annual production of *M. nigricans* in China has continuously grown. In 2021, among the 31 regions nationwide, 29 provinces and cities engaged in the aquaculture of *M. nigricans* (AF 2022). In 2020, farming of *M. nigricans* reached a total output of approximately 619,519 tonnes nationwide, showing a substantial increase of 267,747 tonnes and representing a growth rate of 76.1% compared to 2014. It is expected that there will be major breakthroughs in production over the next 10 years (Bi et al. 2022). Overall, the above figures indicate that both the scale and quantity of farms for *M. nigricans* will continue to expand in the next decade. In the middle and lower reaches of the Changjiang River, especially in Huzhou of Zhejiang Provinces the number of farms is continuously increasing due to the species' widespread popularity (Rural information newspaper 2021; Huzhou Science and Technology Bureau 2023).

The *facility* module assessed the potential risks of *M. nigricans* escaping from aquacultural facilities into the risk assessment area. This module consists of two parts, namely facility and risk of unintentional release of the target or non-target organisms from the facility. The aquacultural facility can very rarely prevent the escape of the target species. The farming of *M. nigricans* in China is quite unique. It takes approximately 1–2 years from seedling cultivation to the provision of mature fish for the market. Because of the different requirements for farming conditions at each stage, larvae are frequently transferred between various farms across the country to reduce costs. Farming technologies for *M. nigricans* are diverse, including intensive pond farming, land-based container farming, cylindrical tube farming, cage culture, and crab-bass polyculture. Many small- and medium-sized farms frequently change their farmed species due to market fluctuations, lacking strict monitoring, farming registration records, and rigorous isolation and disinfection measures. Therefore, *M. nigricans* is highly prone to escaping during farming and transportation. In the *facility* module, the section on release of target organism and farming achieved the highest scores. Although the import section of the *pathway* module was not as high (1.6, medium risk), the overall likelihood and frequency of this species spreading into the wild is predicted to increase.

The *organism* module assessed the potential impacts of *M. nigricans* escaping from its holding facilities. Compared to the other modules, this received the highest overall score. This indicated that the climate of the middle and lower reaches of the Changjiang River matches that of the native range of *M. nigricans*. This species is most abundant in warm eutrophic lakes, rivers, ponds, swamps, reservoirs, and pools of creeks associated with shallow shorelines and submerged structure such as logs, rocks, and aquatic macrophyte beds (Claussen 2015). Invasion by *M. nigricans* occurs mostly in disturbed habitats (Pereira and Vitule 2019). The tributaries of the middle and lower reaches of the Changjiang River are densely distributed with a large human population and a wide range of human activities affecting the rivers in the area. The risk assessment area also features numerous small- to medium-sized reservoirs, with suitable habitat for *M. nigricans* to complete its life cycle. For example, the extensive water system of Lake Taihu in the lower reaches of the Changjiang River, where few natural predators coexist, is an ideal habitat for *M. nigricans*. The eutrophic ecosystem of Lake Taihu provides abundant food for this species, facilitating the successful escape of individuals into the wild (Li et al. 2016). A moderately high risk of dispersal exists, meaning that, once released, *M. nigricans* could spread quickly by natural means throughout the risk assessment area, hence becoming difficult to control.

In the *infectious agent* module, disease and parasites remain a major bottleneck in the development of the aquaculture industry (Madsen and Stauffer 2024). The occurrence of diseases is not solely a problem of single pathogens but is directly related to factors such as breed, feed nutrition, climate, water quality, and farming management. The percentage of high-quality stocks of *M. nigricans* (i.e. of better genetic quality and with no pathogen carriers) is not yet high. Low-quality larvae are highly susceptible to viral infection during farming and transportation, leading to the spread of diseases. Infection of *M. nigricans* is becoming serious. Common diseases during the juvenile stage include those caused by parasitic organisms such as (cupped) ciliates and rotifers. In the adult stage, bacterial diseases like *Nocardia* spp. and *Flavobacterium* have a higher incidence rate.

Viral diseases such as *M. nigricans* Virus and Infectious Spleen and Kidney Necrosis Virus are prevalent and have a high mortality rate. Parasitic diseases are mainly caused by anchor worms. Therefore, despite the current farming situation of *M. nigricans*, moderate risk scores were attributed in each section of the *infectious* agent module. With the expansion of the farming scale, the risk of disease outbreaks in *M. nigricans* will inevitably increase. We recommend that scientists, enterprises, and farmers strengthen information exchange, regularly communicate, assess disease outbreak, reduce disease risks, and minimize economic losses.

The *socio-economic* module consists of three parts: market impact, eradication costs, and impacts at a wider local or national scale. The assessors found the assessment in this module very difficult to conduct because few references (especially quantitative) could be retrieved, whereas available references were mostly within a western context where this species is not important for aquaculture. In such cases, both economic benefits and adverse losses are more likely to occur. Therefore, the confidence level of the responses in this module were mostly the same (i.e. low to medium) and responses lacked critical evaluation and references in support. Overall, the assessors were not familiar with the way the questions were formulated and had to resort to expert opinion ('best guess'). Two of the three assessors even thought this module was not appropriate for the assessment of *M. nigricans* in China, and that it should be redesigned for a Chinese context.

As indicated previously, unlike the broad range of applications of the AS-ISK (Vilizzi et al. 2021), the ENSARS has so far been applied only in Türkiye, Brazil, and China, despite the scheme having been published in 2008 (Copp et al. 2009; Gopp et al. 2016a; Tarkan et al. 2020; de Camargo et al. 2022; Wei et al. 2024). This may be also because of the absence of a convenient electronic tool for the ENSARS as available for the AS-ISK. On the other hand, the requirement for a large volume of knowledge about the species assessed may also represent a limitation for the assessors (de Camargo et al. 2022).

Overall, it is a time-consuming process to obtain reliable results even with a multidisciplinary assessment team, and these results may vary depending on the quality of the supporting information. In China, there is still a lack of research on the impacts of invasive *M. nigricans*, and this may have affected the assessment with a lower confidence level. Although the assessors could indicate their confidence level, the results were still likely to be affected by available information and personal judgement without quantitative justification.

In this study, the original English of the ENSARS text was translated verbatim into simplified Chinese by two authors of this study (HW and SL). Also, all assessments were conducted by definition within a Chinese context. In this regard, the Chinese people tend to have lower levels of probabilistic thinking compared to Western culture, which means our assessors were uncomfortable in providing a confidence rank for the responses (see Copp et al. 2021). At the same time, because of the great and increasing economic benefits of *M. nigricans* in aquaculture in China, the assessors might be biased in providing answers to the questions, which may lead to a negative effect on the aquaculture for this species.

In conclusion, this study has indicated that the ENSARS protocol, supported by the pre- screening with the AS-ISK, can provide useful information for the risk assessment of aquacultural species in China. *Micropterus nigricans* is definitely one of the species that possesses a moderately high risk of invasion and therefore needs to be managed in the middle and lower reaches of the Changjiang River. Our research team carried out field monitoring in Huzhou in 2023, when escaped adults of this species were discovered and recorded. Conduction of regular field surveys is suggested especially in the tributaries of both the Changjiang River and the Qiantangjiang River nearby the farms. Although adapted from an internationally recognised non-native species risk assessment scheme, the ENSARS has been developed within a European context. It is therefore suggested that this scheme should be modified according to a local context if used in other parts of the world.

Authors' contribution:

SL contributed to research conceptualization, methodology, data analysis, data interpretation and original draft writing. HW contributed to methodology, data collection, and draft review & editing. BK, DX, XX contributed to data collection and data interpretation. FL contributed to field investigation, data collection, and data interpretation. JRS contributed to data interpretation, funding provision and draft review and editing. DG contributed to research conceptualization and funding provision.

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Supplementary material

The following supplementary material is available for this article:

Table S1. AS-ISK reports.
Table S2. Assessment results of the ENSARS *pathway* module
Table S3. Assessment results of the ENSARS *facility* module
Table S4. Assessment results of the ENSARS *organism* module
Table S5. Assessment results of the ENSARS *infectious agent* module
Table S6. Assessment results of the ENSARS *socio-economic* module

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